The relationship between right-sided tumour location, tumour microenvironment, systemic inflammation, adjuvant therapy and survival in patients undergoing surgery for colon and rectal cancer

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Introduction

There has been increasing interest in the role of tumour location in the treatment and prognosis of patients with colorectal cancer (CRC), specifically in the adjuvant setting. Together with genomic data this has led to the proposal that right-sided and left-sided tumours should be considered as distinct biological and clinical entities. The aim of the present study was to examine the relationship between tumour location, tumour microenvironment, systemic inflammatory response (SIR), adjuvant chemotherapy and survival in patients undergoing potentially curative surgery for stage I-III colon and rectal cancer.

Methods

Clinicopathological characteristics were extracted from a prospective database. MMR and BRAF status was determined using immunohistochemistry. The tumour microenvironment was assessed using routine H&E pathological sections. SIR was assessed using mGPS, NLR, NPS and LMR.

Results

972 patients were included. The majority were over 65 years (68%), male (55%), TNM stage II/III (82%). 40% of patients had right-sided tumours, 31% had rectal cancers. Right-sided tumour location was associated with, older age (p=0.0001), deficient MMR (p=0.005), higher T stage (p=<0.001), poor tumour differentiation (p=<0.001), venous invasion (p=0.021) and high $CD3^+$ within cancer cell nests (p=0.048). Right-sided location was consistently associated with a high SIR, mGPS (p=<0.001) and NPS (p=<0.001). There was no relationship between tumour location, adjuvant chemotherapy (p=0.632) or cancer-specific survival (CSS) (p=0.377). In those 275 patients who received adjuvant chemotherapy; right-

sided location was not associated with MMR status (p=0.509) but was associated with higher T stage (p=0.001), venous invasion (p=0.036), $CD3^+$ at the invasive margin (p=0.033) and $CD3^+$ within cancer nests (p=0.012). There was no relationship between tumour location, SIR or CSS in the adjuvant group.

Conclusion

Right-sided tumour location was associated with an elevated tumour lymphocytic infiltrate and an elevated SIR. There was no association between tumour location and survival in the non-adjuvant or adjuvant setting in patients undergoing potentially curative surgery for stage I-III colon and rectal cancer.

Introduction

Worldwide, colorectal cancer (CRC) is the third most common cancer in men and the second most common in women with over half of cases occurring in developed nations (Ferlay et al.2012). In the United Kingdom five-year survival for patients diagnosed with CRC is approximately sixty percent (Cancer Research UK, 2011). Several population-based studies and data from clinical trials have reported that primary tumour location provides prognostic value in terms of patient survival. Over the last few decades most epidemiological studies from western populations report a continued rightward shift of colorectal cancer (Fleshner et al. 1989; Cucino et al. 2002; Alley & McNee 1986). Cancers of the right colon are more likely to be diagnosed in patients who are older and female; they are associated with locally advanced tumours, with greater mucinous component, poor differentiation deficient mismatch repair (MMR) and BRAF V600E mutation (Fleshner et al. 1989; Alley & McNee 1986; Weiss et al. 2016; Gelsomino et al. 2016; Clarke & Kopetz 2015).

The clinical and biological distinction between colon and rectal cancer is widely recognised. Although several studies report that right- and left-sided colonic tumours should be considered as a distinct biological entity, the debate about the clinical relevance of this is ongoing. Moreover, the clinical utility of this distinction remains unclear with little evidence of its relevance to patients with stage I-III potentially curable colon cancer in terms of diagnosis, treatment and follow-up. The benefit of fluorouracil based adjuvant therapy in CRC is widely recognised, however in metastatic disease it would appear that right-sided colonic tumours are less responsive to such chemotherapy (Venook et al. 2016). Therefore, a plausible hypothesis is that the prognostic value of tumour location in patients with operable colorectal cancer is dependent on other unmeasured confounding factors. There is a substantial body of evidence which recognises systemic inflammation (Woo et al. 2015; Li et al. 2014) and the tumour microenvironment as important determinants of disease progression

and outcome in both colon and rectal cancer. Inflammation based prognostic scores that evaluate the systemic inflammatory response (Li et al. 2014; Park et al. 2016) and the tumour microenvironment (Roxburgh & McMillan 2012; Klintrup et al. 2005) have yielded prognostic value independent of the widely used TNM staging system and so are candidates as potential confounding factors. The aim of the present study was to examine the relationship between tumour location, tumour microenvironment, systemic inflammation, adjuvant therapy and survival in patients undergoing potentially curative surgery for stage I-III colon and rectal cancer.

Patients and methods

Clinicopathological characteristics

Patients were identified from a prospectively collated database of patients undergoing surgery for colorectal cancer in a single surgical unit at the Glasgow Royal Infirmary between 1997 and 2016. Patient exclusions were based on the following criteria: metastatic disease including those patients with peritoneal involvement, emergency surgery, surgery with palliative intent, surgery for inflammatory bowel disease related malignancy, neoadjuvant chemoradiotherapy (excluded due to the potential immunological impact on the tumour microenvironment), familial cancer syndrome, underlying inflammatory condition or mortality within 30 days of surgery. Patients with tumours proximal to the splenic flexure were considered as right-sided. Tumours were staged according to conventional TNM classification with additional data retrieved from pathological reports issued after resection. Routine pathological elastica staining was used to assess the presence of venous invasion (Roxburgh et al. 2010). Following surgery patients were discussed at a local multi-disciplinary meeting. Those patients undergoing colonic or rectal surgery with stage III or high-risk stage II disease without significant comorbidity, were offered 5- fluorouracil-based adjuvant chemotherapy with or without oxaliplatin, based on guidelines at the time.

Patients were routinely followed up for 5 years after surgery. Date and cause of death was crosschecked with the cancer registration system and the Registrar General (Scotland). Death records were complete until 1st May 2016, this acted as the censor date for survival analysis. Cancer-specific survival (CSS) was measured from date of surgery until date of death from recurrent or metastatic colorectal cancer. Overall survival (OS) was measured from date of surgery until date of death from any cause.

The West of Scotland Research Ethics Committee approved the study.

Assessment of MMR and BRAF status

A subset of the patients in the full cohort underwent evaluation of MMR status, BRAF status and assessment of the tumour microenvironment. Using immunohistochemistry, a previously constructed tissue microarray comprising cores of formalin-fixed paraffin embedded cancer tissue was used to assess MMR and BRAF status. Immunohistochemistry for MMR status was previously described (Park et al. 2016). MMR protein expression was reported as MMR competent or deficient by a single blinded observer.

For assessment of BRAF status tissue microarrays were dewaxed in xylene and rehydrated with graded alcohols. Antigen retrieval was performed using Tris-EDTA buffer at pH9 under pressure for 5 minutes. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide for 10 minutes. 10% Casein was applied for 20 minutes as a blocking solution. Tissue microarrays were incubated overnight at 4^{o}C with anti-human BRAF V600E mouse monoclonal antibody (clone VE1, Spring Biosciences, US) at a concentration of 1:200. After washing in TBS, Envision (Dako) was applied for 30 minutes at room temperature before washing in TBS again. DAB substrate was added for 5 minutes until colour developed before washing in running water for 10 minutes. Slides were then counterstained in haematoxylin for 60 seconds and blued with Scotts' tap water before being dehydrated through a series of graded alcohols. Cover slips were applied using distrene, plasticizer, xylene (DPX). BRAF V600E mutation was reported as absent or present by a single blinded observer.

Assessment of the tumour microenvironment

Assessment of the tumour microenvironment was performed using routine haematoxylin and eosin-stained tissue sections. Klintrup-Mäkinen (KM) score (low/high) and extent of tumour stroma was assessed using tumour stroma percentage (≤50% = low or ≥50% = high), both previously described (Klintrup et al. 2005; Mesker et al. 2007). Tissue sections were also

used to assess lymphocytic tumour infiltrate. Immunohistochemistry for $CD3^+$ (mature) and $CD8^+$ (cytotoxic) T-lymphocytes was performed as per methodology previously described (Richards et al. 2014). T-lymphocyte density at the invasive margin and within the cancer cell nests was semi-quantitatively graded as low or high. Investigators were blinded to clinical data, pathological data and survival outcome.

All cases were co-scored by a second investigator to ensure consistency of scoring.

Assessment of the systemic inflammatory response

Serum CRP, albumin and differential white cell count were measured within 30 days prior to surgery and recorded prospectively. Pre-operative systemic inflammatory responses were defined using the modified Glasgow Prognostic Score (mGPS), the neutrophil:lymphocyte ratio (NLR), the neutrophil:platelet score (NPS) and lymphocyte:monocyte ratio (LMR). The mGPS was constructed as described previously (McMillan, 2013) (patients with CRP ≤10 mg/L scored 0, CRP > 10mg/L scored 1 and CRP > 10mg/L and albumin < 35g/L scored 2). On the basis of previously published thresholds, NLR>5 was considered elevated (Guthrie et al. 2013). The NPS was calculated as previously described (Watt et al. 2015), platelet count<$400x10^9$/L and neutrophil count <$7.5x10^9$/L scored 0, either a neutrophil count>$7.5x10^9$/L or platelet count>$400x10^9$/L scored 1, and those with elevated neutrophils and platelets scored 2. LMR was considered as either low (≤ 2.38) or high (>2.38) as previously described (Chan et al. 2017).

Statistical analysis

The association between tumour location and clinicopathological characteristics, measures of the tumour microenvironment and measures of systemic inflammation were analysed using

the Chi-squared test. 5-year cancer specific and overall survival was examined using Kaplan-Meier log-rank survival analysis and univariate Cox-proportional hazards regression to calculate hazard ratios (HR) and 95% confidence intervals (95% CI). Variables found to be statistically significant ($p<0.05$) on univariate analysis were entered into a Cox regression multivariate model using a backward conditional method. A p value of <0.05 was considered to be significant. Analyses were performed using SPSS software version 21 (IBM SPSS).

Results

Full Cohort

972 patients who underwent elective surgery with curative intent for stage I-III colorectal cancer between January 1997 and 2016 were included in the analysis. Clinical and pathological features are shown in Table 1. Two thirds of the patients included were over the age of sixty-five and 55% were male. 178 (18%) patients had TNM stage I disease, 437 (45%) had stage II disease and 356 (37%) patients had stage III disease. 81 patients (16%) with stage II disease and 196 (45%) patients with stage III disease received adjuvant chemotherapy. In the first decade 24 (12%) patients with stage II and 64 (37%) patients with stage III disease underwent adjuvant chemotherapy. In the latter decade this increased to 57 (18%) patients with stage II and 132 (51%) patients with stage III disease. 389 (40%) patients had tumours located within the right colon, 285 (29%) within the left colon and 298 (31%) had rectal cancer.

In terms of clinicopathological characteristics, right-sided tumour location was associated with older age (p=0.001), higher T stage (p=<0.001), high venous invasion (p=0.021), poor tumour differentiation (p=<0.001) and MMR deficiency (p=0.005) but was not associated with ASA (American Society of Anesthesiology) grade (p=0.088), nodal stage (p=0.320), marginal involvement (p=0.466), tumour necrosis (p=0.422) or BRAF status (p=0.566). Tumour location was not associated with the administration of adjuvant chemotherapy (p=0.632).

When the tumour microenvironment was considered, there was no association between tumour location and Klintrup- Mäkinen Grade (p=0.431) or tumour stroma percentage (p=0.543). Right sided tumour location was associated with high $CD3^+$ within cancer cell

nests (p=0.048) but not at the margin (p=0.160). Right-sided tumour location was not associated with $CD8^+$ within cancer cell nests (p=0.666) or at the tumour margin (p=0.194).

When the preoperative systemic inflammatory response was considered; right-sided tumour location was associated with a greater systemic inflammatory response as measured by the mGPS (p=<0.001) and NPS (p=<0.001). Right-sided tumour location was associated with a low preoperative LMR (p=0.005) (Table 1).

The relationship between right-sided tumour location and the systemic inflammatory response was examined separately in T1/2, T3 and T4 tumours in the whole cohort. In those patients with T1/2 tumours, right-sided tumour location was not associated with mGPS (p=0.404), NLR (p=0.393), NPS (p=0.247) or LMR (p=0.137). In those patients with T3 tumours, right-sided tumour location was directly associated with NPS (p=0.001) but was not associated with mGPS (p=0.119), NLR (p=0.514), or LMR (p=0.299). In those patients with T4 tumours, right-sided tumour location was directly associated with mGPS (p=0.025), NPS (p=0.020) and inversely with LMR (p=0.015).

The median follow-up of survivors at time of censor was 54 months (interquartile range: 28 - 107 months), with 192 cancer-related deaths and 188 non-cancer-related deaths. Right-sided tumour location was not associated with CSS (p=0.377) or OS (p=0.205) (Table 1)

Univariate analysis in colon cancer patients

On univariate analysis in patients with colonic cancer, age (p=0.001), ASA grade (p= <0.001), T stage (p=<0.001), N stage (p=<0.001), venous invasion (p=0.002), $CD3^+$ within nests (p=0.001), mGPS (p=<0.001), NLR (p=0.007) and NPS (p=<0.001) were associated with reduced CSS. Sex, MMR status, tumour differentiation, LMR and tumour location (i.e. left or right colon) were not associated with CSS on univariate analysis. On multivariate

analysis, ASA grade (HR 1.91, p=0.035), N stage (HR 2.96, p=<0.001) and NPS (HR 2.35, p=0.005) were independently associated with reduced CSS. 164 (24%) patients with colon cancer underwent adjuvant chemotherapy. On univariate analysis in this group, T stage (p=0.006), mGPS (p=0.002) and NPS (p=0.010) were associated with reduced CSS. Age, ASA grade, N stage, tumour differentiation, venous invasion, $CD3^+$ within cancer nests, NLR, LMR and tumour location were not associated with CSS on univariate analysis. On multivariate analysis mGPS (HR 1.77, p=0.050) was independently associated with reduced CSS in patients with colon cancer who had undergone adjuvant chemotherapy.

Adjuvant chemotherapy group

There were 275 patients in the adjuvant group who had undergone elective surgery followed by adjuvant chemotherapy with data available regarding tumour subsite. 96 (35%) of those patients had primary tumours located within the right colon, 68 (25%) within the left colon and 111 (40%) within the rectum. Clinicopathological characteristics are shown in Table 2. In terms of patient characteristics, tumour location was not associated with age (p=0.583), sex (p=0.139) or ASA grade (p=0.711). Right-sided tumour location was associated with higher T stage (p=<0.001), poor tumour differentiation (p=0.058) and high venous invasion (p=0.036) but not with nodal stage (p=0.398), margin involvement (p=0.423), tumour necrosis (p=0.779), MMR status (p=0.509) or BRAF status (p=0.460).

When the tumour microenvironment was considered, tumour location was not associated with the Klintrup- Mäkinen Grade (p=0.285) or tumour stroma percentage (p=0.875). Right-sided tumour location was associated with high $CD3^+$ at the margin (p=0.033) and within cancer cells nests (p=0.012) but was not associated with $CD8^+$ density at the margin (p=0.586) or within cancer cell nests (p=0.522).

There was no association between tumour location and the systemic inflammatory response as measured by mGPS (p=0.597), NLR (p=0.183), NPS (p=0.066) or LMR (p=0.128).

The median follow-up of survivors at time of censor was 56 months (interquartile range: 32 - 101), with 60 cancer-related deaths and 27 non-cancer-related deaths. There was no significant difference in terms of CSS (p=0.302) or OS (p=0.076) in the adjuvant group (Table 2).

Discussion

The results of the present study show that although right-sided tumour location was associated with factors pertaining to the host, tumour phenotype and features of the tumour microenvironment, it was not prognostic in terms of survival for patients undergoing surgery for stage I-III colon cancer or indeed for those patients within the adjuvant setting. Therefore, the importance of tumour location as a stratification factor in patients with colorectal cancer may be secondary to such tumour and host factors.

In the present study, right-sided tumour location was associated with advancing age. Also, consistent with previous studies, right-sided tumour location was associated with a greater proportion of T4 tumours, poor tumour differentiation and presence of venous invasion (Weiss et al. 2016; Benedix et al. 2011; Nawa et al. 2008; Benedix et al. 2010). For example, Benedix and co-workers reported that, in 17,641 patients over a three-year period, right-sided colon cancer was more frequently diagnosed in women, older individuals, those with higher ASA grade, locally advanced and lymph node positive disease (Benedix et al. 2010).

In the present study, twenty five percent of right-sided tumours were MMR deficient compared with eleven and eight percent of those tumours located within the left colon and rectum, confirming the findings of other studies which report the association of MMR deficiency with right-sided tumour location (Ward et al. 2001). Surprisingly MMR status was not associated with survival in the full cohort or the adjuvant setting. However, MMR status was only available in a subset of patients (*n*=228). Previous studies have reported the survival benefit conferred by MMR deficiency in the adjuvant setting (Ward et al. 2001; Tejpar et al. 2009; Sinicrope et al. 2013). For example, Sinicrope and co-workers study of 2,580 patients in the adjuvant setting, reported the prognostic impact of MMR deficiency depended on tumour site, where deficient MMR cancers in the right colon had favourable outcomes

compared to those in the left colon (Sinicrope et al. 2011). Therefore, the lack of prognostic value of MMR in the present study is likely due to the small number of patients examined.

In CRC, the local tumour inflammatory response has been reported as having prognostic value independent of tumour stage (Galon et al. 2006; Roxburgh et al. 2009). The relationship between MMR status, systemic inflammation and tumour lymphocytic infiltrate has been examined previously in this cohort. This previous study showed MMR was associated with the local immune tumour infiltrate and the systemic inflammatory response however, MMR had relatively poor prognostic value in comparison to these factors (Park et al. 2016). Our results suggest a significant association between right-sided tumour location and high density of activated T lymphocytes in the whole cohort and in the adjuvant therapy group. This observation may be explained in part by the association of defective mismatch repair with proximal tumour location and in turn with a coordinated, adaptive intratumoural immune response (Park et al. 2016). Nevertheless, the present study is the first to examine the relationship between tumour location and the tumour microenvironment.

The results of the present study showed that for the first time, right-sided tumour location was consistently associated with an elevated systemic inflammatory response as measured by mGPS, NPS and LMR. The basis of this observation is not clear. However, in the present study right-sided tumour location was associated with higher T stage and previous reports suggest higher T stage is associated with increasing systemic inflammatory response hence, T stage may be a potential confounder. Therefore, in the present study, the relationship between right-sided tumour location and the systemic inflammatory response was examined separately in T1/2, T3 and T4 tumours in the whole cohort. In those patients with T1/2 tumours right-sided tumour location was not associated with the systemic inflammatory response. In contrast, in those patients with T3 and T4 tumours, right sided tumour location was associated with the systemic inflammatory response (mGPS, NPS and LMR). Since the

number of patients with T3/T4 tumours was greater in those patients with right-sided tumours it may be that more tumour invasiveness accounted for the association between right-sided tumour location and the systemic inflammatory response and outcome.

In the present study, there was no association between tumour location and CSS or OS in the full cohort or within the adjuvant group. On univariate analysis in patients with colon cancer, tumour location (i.e. right or left) was not associated with CSS or OS or indeed in those patients with colon cancer who had undergone adjuvant chemotherapy. Moreover, results of the present study show that measures of systemic inflammation in patients with colon cancer in the adjuvant and non-adjuvant setting were independently prognostic for CSS. Population-based studies (Meguid et al. 2008; Benedix et al. 2010), and outcomes of retrospectively analysed oncological trials of patients with metastatic CRC (Loupakis et al. 2015; Venook et al. 2016) have reported an increase in mortality for right-sided cancers however, results are conflicting (Weiss et al. 2016; Warschkow et al. 2016). Moreover, they did not adjust for confounders such as the systemic inflammatory response. Therefore, it is of interest that Renfro and co-workers reported that, in more than 22,000 patients from 28 randomised clinical trials of patients with metastatic colorectal cancer, an elevated systemic inflammatory response as evidenced by an absolute neutrophil count and the derived NLR was associated with early mortality whereas KRAS status, patient sex, individual sites of metastases, location of primary tumour (colon v rectum), and prior chemotherapy use did not appear to play a prognostic role (Renfro et al.2017). Additionally, a study evaluating the prognostic role of tumour location in stage III colon cancer patients (PETACC-8 trial) in the context of molecular markers reported right-sided tumour location was not associated with disease-free survival but was associated with shorter survival after relapse when disease became metastatic and with overall survival in both MSI-stable and unstable patients (Taieb et al.

2017); However, potential confounding factors such as the systemic inflammatory response was not taken into account.

A potential limitation of the present study was that only 45% of patients with stage III colorectal cancer received adjuvant therapy. It is well recognised Glasgow Royal Infirmary serves an area of multiple deprivation. As a consequence, many patients have multiple comorbidities and this precludes the use of chemotherapy. Moreover, as the present study spans a period between 1997 and 2016, a period effect should be appreciated. In the present study this effect was demonstrated by the increase in the number of patients with stage III colorectal cancer who received adjuvant chemotherapy from 37% in the first decade to 51% in the latter decade.

In conclusion, right-sided tumour location was associated with host characteristics, features of the tumour microenvironment and the systemic inflammatory response, but not survival in patients undergoing potentially curative surgery for stage I-III CRC. Future studies investigating the role of tumour location in a prognostic or predictive capacity should be aware of the potential confounding role that inflammatory responses may have in these patients.

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Figure legends

Table 1. Associations between right/left/rectal tumours and clinicopathological characteristic, systemic inflammation and the tumour microenvironment in patients undergoing elective surgery for stage I-III colorectal cancer

Table 2. Associations between right/left/rectal tumours and clinicopathological characteristic, systemic inflammation and the tumour microenvironment in patients who have undergone adjuvant chemotherapy following elective surgery for stage I-III colorectal cancer

Table 1. Associations between right/left/rectal tumours and clinicopathological characteristic, systemic inflammation and the tumour microenvironment in patients undergoing elective surgery for stage I-III colorectal cancer

		All ***n*=972 (%)**	**Right** **n=389 (%)**	**Left** **n=285 (%)**	**Rectal** **n=298 (%)**	***p***
Host Characteristics						
Age (n=972)	**<65**	314 (32)	101 (26)	101 (36)	112 (38)	0.001
	65-74	343 (35)	135 (35)	98 (34)	110 (37)	
	>75	315 (33)	153 (39)	86 (30)	76 (25)	
Sex (n=972)	**Female**	438 (45)	188 (48)	132 (46)	118 (40)	0.065
	Male	534 (55)	201 (52)	153 (54)	180 (60)	
ASA status* (n=907)	**1**	162 (18)	52 (14)	48 (19)	90 (23)	0.088
	2	406 (45)	164 (44)	113 (44)	179 (46)	
	3	302 (33)	138 (37)	82 (32)	115 (29)	
	4	37 (4)	17 (5)	12 (5)	9 (2)	
Tumour Characteristics						
T stage (n=969)	**1-2**	207 (21)	57 (15)	65 (23)	85 (29)	<0.001
	3	531 (55)	216 (56)	150 (53)	165 (55)	
	4	231 (24)	115 (29)	69 (24)	47 (16)	
N stage (n=971)	**0**	617 (64)	248 (64)	192 (67)	177 (59)	0.320
	1	259 (26)	101 (26)	71 (25)	87 (29)	
	2	95 (10)	39 (10)	22 (8)	34 (11)	

TNM stage (n=971)	**I** **II** **III**	178 (18) 437 (45) 356 (37)	53 (14) 194 (50) 142 (36)	58 (20) 134 (47) 93 (33)	67 (23) 109 (37) 121 (40)	0.002
Tumour differentiation (n=961)	**Mod/well** **Poor**	870 (90) 91 (10)	323 (84) 62 (16)	272 (96) 9 (4)	275 (93) 20 (7)	<0.001
Venous invasion (n=971)	**No** **Yes**	486 (50) 485 (50)	180 (46) 208 (54)	162 (57) 123 (43)	144 (48) 154 (52)	0.021
Margin involvement (n=971	**No** **Yes**	919 (95) 52 (5)	370 (95) 18 (5)	271 (95) 14 (5)	278 (93) 20 (7)	0.466
Necrosis	**Absent** **Present**	191 (58) 138 (42)	70 (58) 51 (42)	57 (54) 49 (46)	64 (63) 38 (37)	0.422
MMR (n=228)	**Competent** **Deficient**	193 (85) 35 (15)	65 (75) 22 (25)	57 (89) 7 (11)	71 (92) 6 (8)	0.005
BRAF (n=221)	**Wild-type** **V600E mutation**	169 (77) 52 (23)	61 (73) 23 (27)	50 (78) 14 (22)	58 (80) 15 (20)	0.566
Tumour microenvironment						
Klintrup-Mäkinen grade (n=353)	**Weak** **Strong**	233 (66) 120 (34)	88 (70) 37 (30)	73 (64) 42 (36)	72 (64) 41 (36)	0.431

Tumour stroma percentage (n=302)	**Low** **High**	228 (76) 74 (24)	92 (78) 26 (22)	71 (76) 22 (24)	65 (71) 26 (29)	0.543
CD3$^+$ margin density (n=260)	**Low** **High**	148 (57) 112 (43)	54 (59) 38 (41)	36 (48) 39 (52)	58 (62) 35 (38)	0.160
CD3$^+$ cancer cell nest density (n=270)	**Low** **High**	173 (64) 97 (36)	55 (57) 42 (43)	47 (62) 29 (38)	83 (74) 29 (26)	0.048
CD8$^+$ margin density (n=257)	**Low** **High**	150 (58) 107 (42)	53 (60) 35 (40)	38 (50) 38 (50)	59 (63) 34 (37)	0.194
CD8$^+$ cancer cell nest density (n=266)	**Low** **High**	185 (70) 81 (30)	63 (68) 30 (32)	52 (68) 25 (32)	70 (73) 26 (27)	0.666
Systemic inflammation						
mGPS (n=926)	**0** **1** **2**	658 (71) 163 (18) 105 (11)	235 (64) 69 (19) 62 (17)	201 (75) 44 (16) 25 (9)	222 (77) 50 (17) 18 (6)	<0.001
Neutrophil:Lymphocyte ratio (n=814)	**Low** **High**	708 (87) 106 (13)	283 (85) 51 (15)	201 (90) 23 (10)	224 (88) 32 (12)	0.214
Neutrophil:Platelet score (n=817)	**0** **1** **2**	667 (82) 116 (14) 34 (4)	239 (72) 71 (21) 23 (7)	204 (87) 23 (10) 7 (3)	224 (90) 22 (8) 4 (2)	<0.001
Lymphocyte:monocyte ratio	**Low**	155 (16)	80 (21)	39 (14)	36 (12)	0.005

(n=972)	**High**	817 (84)	309 (79)	246 (86)	262 (88)	
Adjuvant therapy (n=970) 1997-2006 2007-2016	 No Yes No Yes	 286 (78) 86 (22) 409 (68) 189 (32)	 107 (84) 21 (16) 184 (61) 75 (29)	 92 (78) 26 (22) 125 (75) 42 (25)	 87 (69) 39 (31) 100 (58) 72 (42)	 0.632
5-year CSS % (SE)			79 (3)	83 (3)	76 (3)	0.377
5-year OS % (SE)			66 (3)	71 (3)	67 (3)	0.205

***ASA: American Society of Anesthesiology physical status classification**

Table 2. Associations between right/left/rectal tumours and clinicopathological characteristic, systemic inflammation and the tumour microenvironment in patients who have undergone adjuvant chemotherapy following elective surgery for stage I-III colorectal cancer

		All *n*=275 (%)	Right n=96 (%)	Left n=68 (%)	Rectal n=111 (%)	*p*
Host Characteristics						
Age (n=275)	**<65**	144 (52)	44 (46)	39 (57)	61 (55)	0.583
	65-74	101 (37)	39 (41)	23 (34)	39 (35)	
	>75	30 (11)	13 (13)	6 (9)	11 (10)	
Sex (n=275)	**Female**	121 (44)	43 (45)	36 (53)	42 (38)	0.139
	Male	154 (56)	53 (55)	32 (47)	69 (62)	
ASA status* (n=256)	**1**	70 (27)	23 (25)	20 (32)	27 (27)	0.711
	2	119 (47)	46 (50)	29 (46)	44 (44)	
	3	62 (24)	20 (22)	13 (20)	29 (28)	
	4	5 (2)	3 (3)	1 (2)	1 (1)	
Tumour Characteristics						
T stage (n=275)	**1-2**	22 (9)	1 (1)	8 (12)	13 (12)	
	3	163 (59)	50 (52)	37 (54)	76 (68)	<0.001
	4	90 (32)	45 (47)	23 (34)	22 (20)	
N stage (n=275)	**0**	85 (31)	31 (32)	23 (34)	37 (28)	0.398
	1	134 (49)	46 (48)	3 (53)	52 (47)	

	2	56 (20)	19 (20)	9 (13)	28 (25)	
TNM stage (n=275)	**I** **II** **III**	3 (1) 81 (30) 191 (70)	0 (0) 30 (31) 66 (69)	0 (0) 23 (34) 45 (66)	3 (3) 28 (25) 80 (72)	0.141
Tumour differentiation (n=274)	**Mod/well** **Poor**	244 (89) 30 (11)	81 (84) 15 (16)	65 (96) 3 (4)	98 (89) 12 (11)	0.058
Venous invasion (n=275)	**No** **Yes**	92 (34) 183 (66)	23 (24) 73 (76)	24 (35) 44 (65)	45 (41) 66 (59)	0.036
Margin involvement (n=275)	**No** **Yes**	246 (90) 29 (10)	88 (92) 8 (8)	62 (91) 6 (9)	96 (87) 15 (13)	0.423
Necrosis (n=87)	**Absent** **Present**	47 (54) 40 (46)	11 (48) 12 (52)	16 (57) 12 (43)	20 (56) 16 (44)	0.779
MMR (n=66)	**Competent** **Deficient**	58 (88) 8 (12)	19 (86) 3 (14)	18 (95) 1 (5)	21 (84) 4 (16)	0.509
BRAF (n=62)	**Wild-type** **V600E mutatation**	46 (74) 16 (26)	15 (75) 5 (25)	13 (65) 7 (35)	18 (82) 4 (18)	0.460
Tumour microenvironment						
Klintrup-Mäkinen grade (n=102)	**Weak** **Strong**	30 (29) 72 (71)	10 (40) 15 (60)	10 (31) 22 (69)	10 (22) 35 (78)	0.285

Tumour stroma percentage (n=79)	**Low** **High**	54 (68) 25 (32)	15 (68) 7 (32)	18 (72) 7 (28)	21 (66) 11 (34)	0.875
CD3$^+$ margin density (n=71)	**Low** **High**	42 (59) 29 (41)	9 (47) 10 (53)	10 (46) 12 (54)	23 (77) 7 (23)	0.033
CD3$^+$ cancer cell nest density (n=73)	**Low** **High**	58 (80) 15 (20)	12 (60) 8 (40)	17 (77) 5 (23)	29 (94) 2 (6)	0.012
CD8$^+$ margin density (n=71)	**Low** **High**	37 (52) 34 (48)	11 (58) 8 (42)	9 (43) 12 (57)	17 (55) 14 (45)	0.586
CD8$^+$ cancer cell nest density (n=73)	**Low** **High**	55 (75) 18 (25)	16 (76) 5 (24)	14 (67) 7 (33)	25 (81) 6 (19)	0.522
Systemic inflammation						
mGPS (n=257)	**0** **1** **2**	190 (74) 47 (18) 20 (8)	56 (68) 19 (23) 8 (9)	49 (75) 11 (17) 5 (8)	85 (78) 17 (16) 7 (6)	0.597
Neutrophil:Lymphocyte ratio (n=249)	**Low** **High**	217 (87) 32 (13)	80 (88) 11 (12)	53 (93) 4 (7)	84 (83) 17 (17)	0.183
Neutrophil:Platelet score (n=239)	**0** **1** **2**	189 (79) 40 (17) 10 (4)	63 (70) 23 (26) 4 (4)	46 (85) 5 (9) 3 (6)	80 (84) 12 (13) 3 (3)	0.066

Lymphocyte:monocyte ratio (n=275)	**Low** **High**	39 (14) 236 (86)	17 (18) 79 (82)	5 (7) 63 (93)	17 (15) 94 (85)	0.128
5-year CSS % (SE)			80 (5)	85 (5)	7 (5)	0.302
5-year OS % (SE)			74 (5)	85 (5)	66 (5)	0.076

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